Applying Helium Ion Microscopy to Study Alport Syndrome in Mice

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The kidney is a vital organ that performs the essential function of maintaining volume, electrolyte and acid-base balance in mammals. The kidney filters approximately 180 liters of blood every day through a complex glomerular filtration barrier. This filtration apparatus consists of highly specialized podocytes, endothelial cells and glomerular basement membrane (GBM), and is characterized by a highly sophisticated three-dimensional architecture. Studying the ultrastructure of the kidney glomerulus by transmission and conventional Scanning Electron Microscopy (SEM) has provided important insights into glomerular biology, pathophysiology, and the underlying mechanisms of kidney diseases. Biopsied kidney has been examined routinely by transmission electron microscopy (TEM) and conventional SEM to identify and classify various glomerular diseases. For example, detecting fusion and effacement of podocyte foot processes by TEM and SEM constitutes a hallmark of proteinuric glomerulopathy/podocytopathy.

In this new era of discovery, remarkable progress has been made in the cell and molecular biology of kidney diseases. This highlights the need for more powerful microscopic techniques to enable the detection of sophisticated cellular and/or molecular events, and possibly to characterize molecular anatomic details of cells and subcellular structures at nanometer resolution scale. Conventional microscopic techniques are no longer able to meet the demands and fill in knowledge gaps. Excitingly, the recently developed high resolution Helium Ion Microscopy (HIM) offers unique advantages over conventional SEM through reduced sample charging, minimizing sample damage, and providing better surface contrast without metal coating. More importantly, it enables an increased depth of field and potentially 5 angstrom imaging resolution. We report here the application of HIM to examine glomerular abnormalities in the collagen type IV α3 chain (Col4a3, an essential component of the GBM) deficient mice that model Alport syndrome. Our study reveals unprecedented details of glomerular abnormalities in Col4a3 mutants including distorted podocyte morphology and an altered glomerular endothelium with disrupted sub-endothelial integrity. More importantly, we were able to clearly visualize the complex, three-dimensional podocyte and endothelial interface by HIM (Fig 1)[1]. Our study demonstrates that HIM provides nanometer resolution to uncover and rediscover critical ultrastructural characteristics of the glomerulopathy in Col4a3 mutant mice.

[1] K. Tsuji, H. Suleiman, J. H. Miner, J.A. Daley, D.E. Capen, T.G. Paunescu, H.A.J. Lu, Scientific Reports 7:11696 (2017).

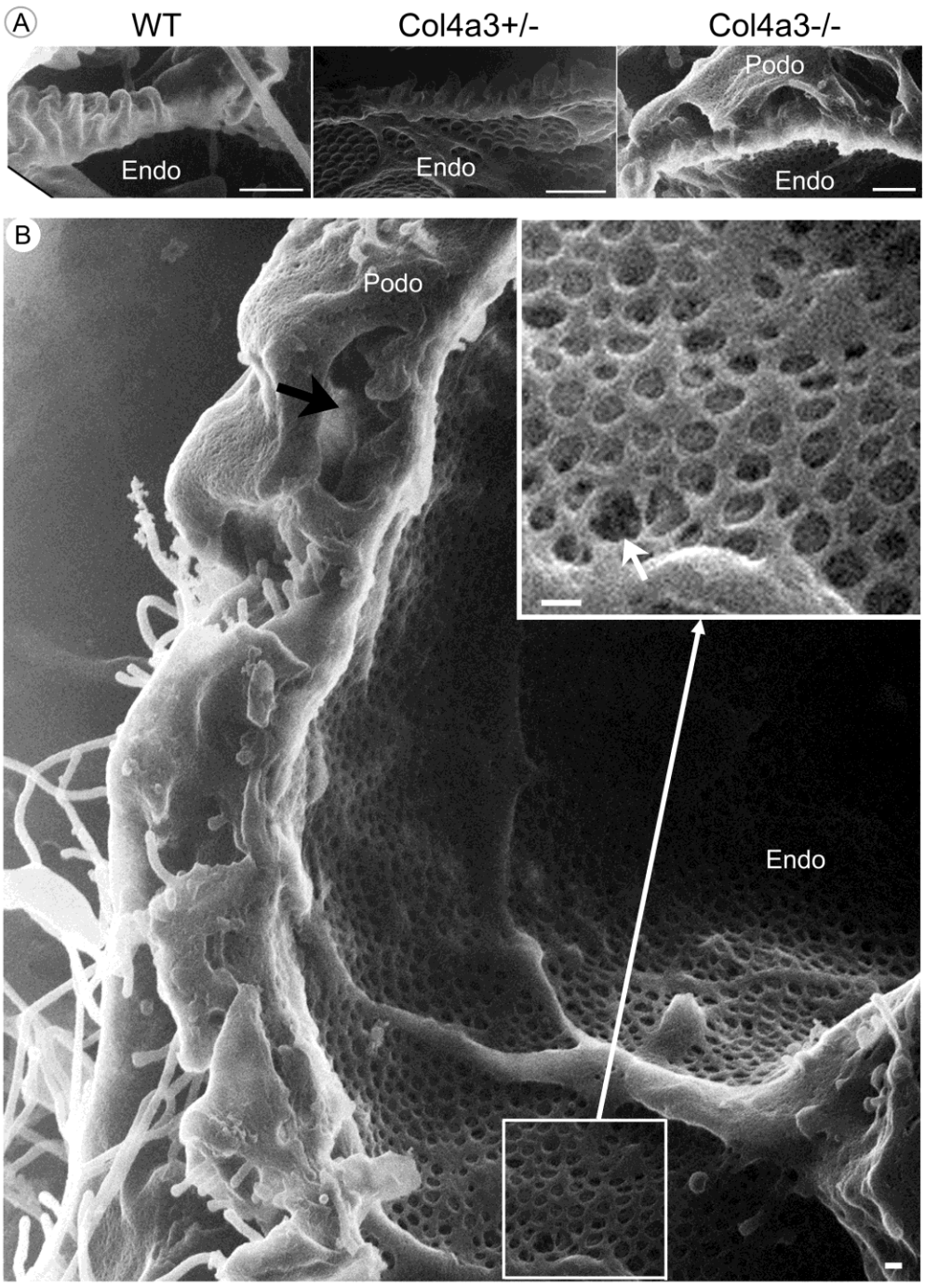

Figure 1. (A) HIM images of the interface between podocytes and endothelium in wild-type (WT), Col4a3+/-, and Col4a3-/- mice. The WT image shows well organized foot processes lining the capillary loops. A similar pattern is seen in Col4a3+/- mice. In Col4a3-/- mice, foot processes appear effaced and form flat sheets covering the GBM. Scale bars, 500 nm. (B) Transverse image of the interface between podocytes and endothelium in Col4a3-/- kidney shows largely effaced foot processes and bridging process structures. The bridging processes and flattened podocyte cell body arch over effaced foot processes (black arrow). The disappearance or fragmentation of the diaphragm-like structure can be seen underneath the fenestrae (white arrow). Scale bar, 100 nm.